PROTOCOL FOR THE EXAMINATION OF VALUE FOR CULTIVATION AND USE OF

FIBRE HEMP VARIETIES

In The Netherlands

2025

Raad voor plantenrassen (Rvp)
Plant Variety Board

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Contents

1. Introduction

This protocol sets out the procedures to be used for the examination of the Value for Cultivation and Use (VCU) of fibre hemp in the Netherlands.

VCU testing of Fibre Hamp consists of:

- Two years of official trials for varieties to be included in the National List (NL1 and NL2)
- Determination of THC content being part of the official testing in any case (Annex 1)

This protocol is based on the assumption of sufficient basic knowledge of experimental techniques, the husbandry of hemp and the processing of hemp. Commonly used methods and treatments are not explicitly described. Unless otherwise indicated it is assumed that the agronomy and the processing of hemp should follow the best local practice of an average Dutch arable farm.

After NL2 the *Raad voor plantenrassen* (Rvp; Plant Variety Board) decides whether or not the variety can be included in the National List based on the VCU results. Varieties included in the National List are approved for marketing.

The VCU of Hemp is not collectively financed (i.e. by breeders, growers and industry together). The testing is organised by the breeders themselves, supervised by Naktuinbouw.

See annex 2 for contact details.

2. Examination of Value for Cultivation and Use of Fibre Hemp

2.1. Trial seed

For all trials, the Trials Coordinator (i.e. the company organizing the trials) makes an inventory of the quantity of seed required of each variety and distributes the seed among the Trial Operators. The breeder (i.e. the applicant of a variety to be tested) submits the amount of seed, untreated, together with information regarding the germination rate (minimum 80%) and the thousand seed weight. The seed of the standard varieties is obtained directly from the breeder/maintainer of the variety concerned.

The Trials Coordinator distributes the seed among the Trial Operators together with a list specifying the thousand seed weights and the trial plan.

The identity of the seed to be used in the trials is verified by Naktuinbouw each year. Before 1 April a minimum of 50 grams of seed of each variety needs to be submitted to:

NAK seed testing station
Att. W. van der Kooij
Johannes Postweg 1
8309 PE Tollebeek, NL

2.2. Trial design

The testing comprises 2 sites per year, preferably located in different regions (sandy and reclaimed peat soils). At least one trial needs to be planted in the north eastern part of The Netherlands (the main growing area).
All trials are carried out in 4 complete replicates.
The varieties of each replicate should be grown in a single lane. A discard plot needs to be planted next to the first and the last plot in a lane. The plot area must be at least 25 m^2.
The plots should be at least 3 m wide and their length must be at least 3 times their width.
The distance between the rows is 12,5 cm.

2.3. Varieties to be tested

<u>New varieties</u>
In the trials a maximum of 10 new varieties of fibre hemp can be tested next to 3 standard varieties. Feminized varieties can be tested as well. Varieties to be admitted to the trials is decided in consultation between the Trials Coordinator and the persons responsible of the (new) varieties.

<u>Standard varieties</u>
Three widely grown varieties in The Netherlands, are taken up in the trials as standard varieties: Uso-31, Futura 75 and Félina 32.

2.4. Trial layout, Trial operations and Trial husbandry

The trial plan is a randomised complete or incomplete block design with discard plots at the front- and backside of the lanes. The varieties of each replicate should be grown in a single lane. The trial should preferably be entirely surrounded by a commercial crop of fibre hemp.
The trial plans are set up by the Trials Coordinator and sent to the Trial Operators together with the trial seed. The fields to be used for the trials should be as regular as possible and, as far as is known, free from *Sclerotinia* and *Botrytis.* The fields must be uniform, or must have undergone treatment to make them uniform without any after-effects. In case of drained fields, the trial lanes must run parallel to the drains and the plots must be cross-drilled to the direction of the drains. Treatments and husbandry must be done in the direction of the trial lanes as much as possible. Furthermore the agronomy should follow best local practice of an average Dutch arable farm.

Seed-propagated varieties must be sown from the middle of April, as soon as soil conditions permit. The target plant population is 200 plants/m^2 after emergence. The seed rate is corrected for thousand seed weight and germination rate. The distance between the rows is 12,5 cm and the sowing depth is 1 – 2 cm.

There should be no large differences in plant population directly after emergence. In the event of more than 20% difference in plant population between plots, plots need to be thinned to a regular stand. Plots should not be thinned to less than 150 plants /m^2. Plots with less than 120 plants/m^2 are excluded from further testing.
Fertilisation depends on the soil fertility of the trial field and the expected yield level. As a guideline the crop must be supplied with approx.. 12 kg Nitrogen, 15 kg Potassium (K2O) and 4 kg Phosphate (P2O5) per Mton of aboveground dry matter yield.

2.5. Observations and measurements during the growing season

2.5.1. Data recording

Field observations and measurements are carried out by the Trial Operator. In addition, the Trials Organiser and Naktuinbouw can make random observations also. The observations should be recorded electronically or in writing in the format agreed by the Trials Coordinator. The plot records should be sent to the Trials Coordinator at once, i.e. as soon as possible after harvest. Observations and measurements in the plot should be made around the area to be harvested (see 2.6: harvest), such that the crop on the part that is going to be harvested will not be disturbed.

2.5.2. Characteristics

The following characteristics are observed by the Trial Operator, whereby the Trial Operator should indicate which scale of observation has been used.

Characteristics should be scored at the widest possible range of scores on a scale from 1-9.
A high score implies a positive assessment of the characteristic concerned and a low score a negative assessment. In addition, the highest and the lowest observation needs to be described.

Plant population after emergence

Shortly after emergence 3 x 0.5 m^2 per plot (3 rows over a length of 1 metre at 3 different spots in the plot) must be counted. The counting is converted into number of plants per m^2.

Earliness of development

About three weeks after emergence (when the earliest variety has reached ground cover of 75%) the earliness of development is recorded as a score (9 = very early development, 1 = very late development). This observation is repeated once or twice until ground cover of all varieties is complete.

Median flowering date

The time of flowering determines the length of the growing period. Early flowering is preferred as early flowering varieties have a higher harvest security.

The flowering date is based on the male flowers as the start of female flowering is difficult to be observed. The time of flowering must be determined per plot as the day on which 50% of the (male or monoecious) plants are flowering. The time of flowering is expressed as the number of days after sowing.

Standing ability (or lodging) at different growth stages

In the event of lodging, the observations are recorded as a score. The observations must be recorded on a scale of 1-9, with 9 representing the lowest amount of lodging (good standing ability) and 1 representing the highest amount of lodging (poor standing ability).

The first observation must be made immediately after lodging and repeated if further lodging develops. The last observation must be made just before harvest.

In addition to the scores, the degree of lodging in the plot with the most amount of lodging and of that in the plot with the least amount of lodging must be described as a lodging percentage.

A plant is considered weak if it bends over more than 45° out of the row or if it is buckled.

Proportion of male plants

The number of male plants is assessed just before harvest and recorded as a percentage of the total number of plants.

Disease observations

Possible disease infections must be recorded as a score (9 = resistant = very low % of infection, 1 = highly infected). The disease and the degree of infection must be described.

Plant height

The length is to be measured at harvest in cms per plot

Other observations

Any other observations that may be of importance in examining the trial must be made, for example in the case of irregularities in the trial (soil structure effects or fertility differences), damage to any of the plots, damage caused by drought, diseases, losses during harvest, etc.

2.6. Harvest and processing

2.6.1. Determination of THC content

According to Annex I of the EC Regulation No. 1122/2009 the sampling for the determination of the THC content must be done at daylight in the period running from 20 days after the start of flowering to 10 days after the end of flowering. This is at the time when at least 50% of the seeds have reached their final shape and size, which is also the criterion for the time of harvest. The THC sample must be taken just before harvest. The time of harvest can be different per variety. Samples for THC content should be taken by the Trial Operator from one trial. Naktuinbouw, in consultation with the Trial Operator, determines which trial should be used for sampling. Prior to the sampling the Trial Operator should inform Naktuinbouw about the time of sampling.
In addition Naktuinbouw takes samples from the trial for authentication of the VCU samples. The costs involved with the determination of the THC content from both trials shall be borne by the applicants of the varieties applied for National Listing.

Sampling
A 30 cm long upper part of the stem is harvested per plot from 20 randomly chosen plants spread across the plot (excluding the edges of the plot). Male plants should be excluded from the sample and the sample should be representative for the whole plot. Each sample shall be placed in a spacious cotton bag and dried within 24 hours, by an institution designated for drying, storing and processing of the samples.

Analysis of the samples
The samples are dried directly in the cotton bags in a drying oven during 72 hours at 35 °C. After drying until the moment of further analysis the samples are kept in a dark store at 15 ºC and 15% relative humidity.
The THC content is determined by Wageningen Food Safety Research (WFSR) by use of gas chromatography. The samples are prepared as follows:
The dried upper parts of the stem are crushed in the bag. The rough material is removed by means of a round sieve with gaps of 5,5 mm. The remaining fine material is screened on a split sieve with gaps of 2,1 mm and a round sieve with gaps of 2,05 mm respectively. The four replications of each variety per trial are combined into one mixed sample from which a representative sub-sample is taken and placed in a WFSR sampling jar together with an identifying label to be delivered at the laboratory of WFSR for a quantitative analysis of the THC content. The THC content is expressed in grams per 100 gram of the laboratory sample dried to constant mass.

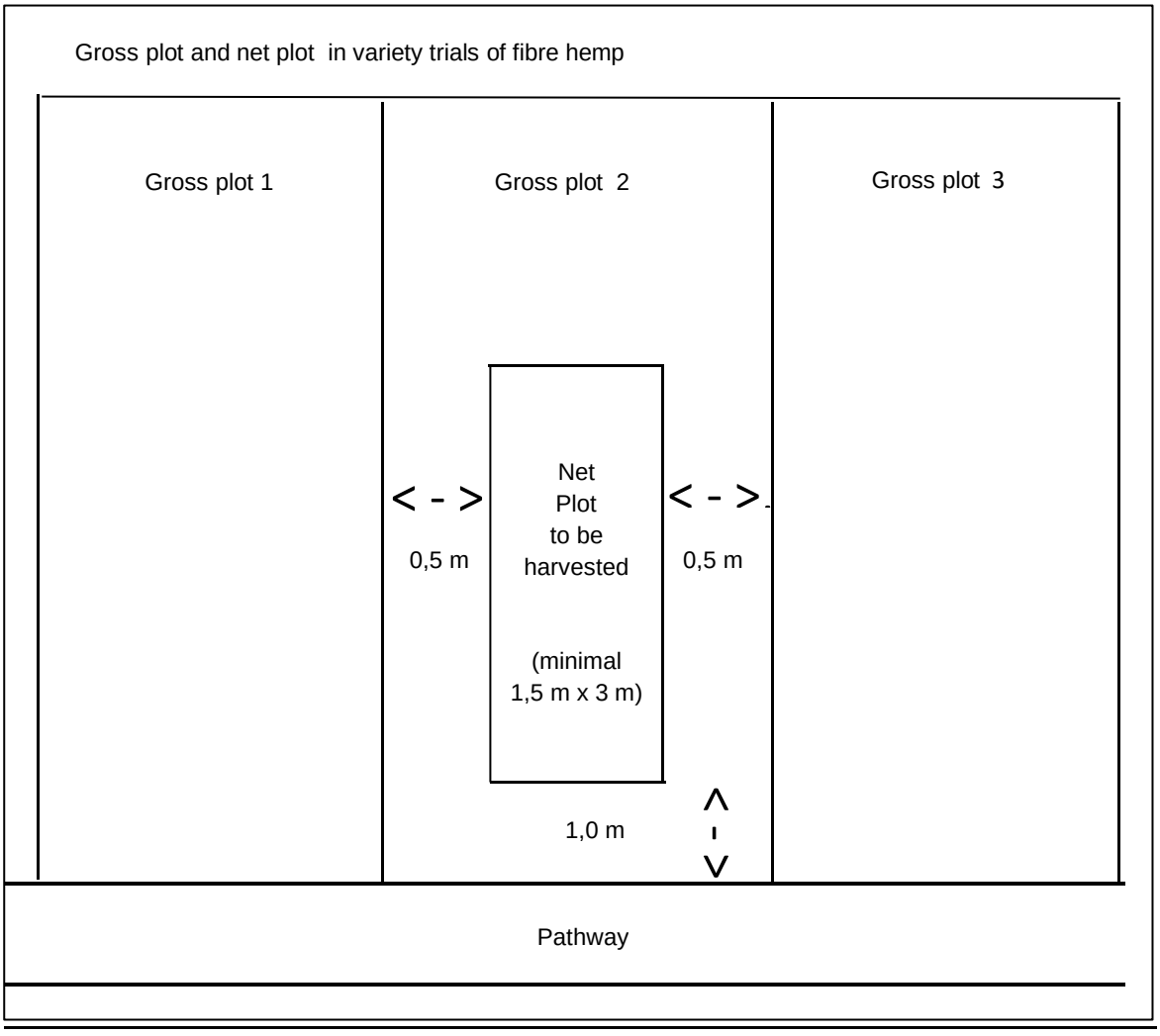
2.6.2. Determination of the yield

Harvest time
Each variety is harvested at its own maturity due to the usually large differences in time of maturity. Each variety is harvested at the time when at least 50% of the seeds have reached their final shape and size, on average over all four replicates. At that time three quarters of the plants contain no flowers anymore and seeds are clearly visible in the top of the stems. This is the time when the samples for yield are taken. All four replicates of a variety are harvested at the same time.

Harvest

A net plot area of at least 4,5 m^2 (1,5 m x 3 m minimal length) is harvested per plot. The total number of stems is bundled (bundles should not be too thick for drying), weighed, transported and dried on a drying floor or in drying bins during about 5-14 days until the material has reached a constant moisture content. The dry bundles are weighed and the dry weights are converted into tons dry matter per ha.

In order to avoid side effects, at least one meter from the end of the plot should be left unharvested when this end is beside the pathway and at least 0,5 meters from the side of the plot should be left unharvested when this side is adjacent to another plot.



2.7. Trial Log

All the operations described above, plus any irregularities or unforeseen matters that may affect the trial results must be recorded in a trial log and must be submitted to the Trials Coordinator.

Annex 1.

Short guideline for the harvest timing of fibre hemp

Hemp is dioecious by nature, but most varieties are monoecious by breeding: i.e. female flowers occur together with male flowers on a plant. However, most varieties are rarely 100% monoecious. The share of male plants can vary due to segregation in successive generations. Male plants show early senescence and do not contribute to the yield in general.
Uso 31 is monoecious. Chamaeleon and Enectarol are dioecious.
The female flower can be recognized at flowering by the white or pinkish white very small stigmata and on their position in the top of the shoots.
The male flowers are much bigger than the female flowers and can be recognized by their white to light green (sometimes light purple) color.
Male flowers do not occur in the top of monoecious plants.
Seeds start developing directly after pollination. The seed is enclosed by husks.

Note: completely male flowering plants are disregarded in the assessment of the harvest time.

Definitions:

Start of flowering	the tops are densely covered with female flowers without seeds in their early stages of development..
Flowering halfway	the tops are still densely covered with flowers, but seeds start developing at the lower end of the top.
Flowering at 3/4 stage	some tops have finished flowering with some viable flowers remaining, together with considerable amounts of seeds.
Flowering finished	developing seeds can be found up to the top, which is not dense anymore and individual seeds are visible up to the top of the plant.

Assessment of the harvest time

The time when at least 50% of the seeds have reached their final shape and size is the criterion for the time of harvest. This condition is fulfilled when the seeds have reached the milk stage of development. (the dehusked seed can easily be crushed between the fingers, squeezing out a fluid content).

This means in practice that there are several plants in a field which have finished flowering already at the time when the criterion is met.

A representative number of 30 tops are cut from the field. These tops are laid out on the ground to determine whether the sample is sufficiently uniform. If not, a new sample needs to be cut.
The number of flowering tops in this sample needs to be observed (see definitions above).
The 50% criterion is met and the variety is ready for harvest when the majority (about three quarter) of the tops contains no flowers anymore and when seeds are clearly visible in the top of the plants.

Annex 2: Contact details

Raad voor plantenrassen (Rvp) / Naktuinbouw
Plant Variety Board / Naktuinbouw

Postbus 40
2370 AA Roelofarendsveen, NL

Visitors address:
Sotaweg 22
2371 GD Roelofarendsveen, NL

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www.naktuinbouw.nl
www.plantenrassen.nl

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Naktuinbouw – Authentication Service

Johannes Postweg 1
8309 PE Tollebeek, NL

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